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STUDIES OF ENVIRONMENTAL FATES OF DIMP AND DCPD

Final Report

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The objectives of this study were to determine the rates of microbial and photochemical transformations of DCPD and DIMP in the soils and waters of Rocky Mountain Arsenal.

Biotransformation of DCPD was observed to occur very slowly in soil and water. DIMP underwent biotransformation slowly in soil only. DCPD was observed to photolyze in natural waters by an indirect mechanism, and DIMP was observed to be non-photoreactive.

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EXECUTIVE SUMMARY

The role of photochemical and microbiological transformation processes in determining the environmental fates of diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) was investigated.

DIMP was unreactive to both direct and indirect photolysis in distilled and natural waters. This indicates that photolysis is not an important process for DIMP in aquatic systems. Biotransformation of DIMP also was not observed in natural waters after we attempted to acclimate microorganisms. Biotransformation of DIMP was slow in soil at 25°C and nearly nonexistent at 10°C. A half-life of more than 2 years is predicted for the biotransformation of DIMP to CO_2 in soil.

DCPD underwent photolysis only in the presence of natural water sensitizers. A half-life of more than 76 days was estimated. Biotransformation of DCPD was very slow in both soil and natural waters. Environmental half-lives for conversion to CO_2 by biotransformation were estimated to be 4 to 7 years in soil and 1 to 2 years in water at 25°C. Volatilization of DCPD from water was an important transport process, and a half-life of 5 days was estimated.

ACKNOWLEDGMENTS

We are indebted to our support staff for their assistance throughout the course of this project. In particular, our thanks go to Mr. Bradford Gibson and Ms. Clair Ingersoll for chemical analyses; Mr. Norman Kirshen for photolysis studies; and Ms. Barbara Mason, Nancy Bauer, and Mr. David Osugi for biodegradation studies. We are also indebted to Dr. James H. Smith and Ms. Ann Neville for volatilization studies.

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I INTRODUCTION

Rocky Mountain Arsenal (RMA) has long been used for the production of chemicals for both military and agricultural use. Over the years, these chemicals and their precursors, intermediates, and residues, have been disposed of on land or in permeable basins. The adverse effects of this disposal method are becoming apparent; the groundwater beyond the boundaries of RMA is contaminated.

The Installation Restoration Program of the U.S. Army is designed to assess problems resulting from land disposal at Army installations and to take action to prevent contamination from becoming a health or environmental hazard off post. To assist the Army in making these decisions, we estimated the persistence of two RMA groundwater contaminants, diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) with respect to biological and photochemical transformation processes that may occur at RMA.

II BACKGROUND

Biodegradation is important for the transformation of many chemicals in the environment. Microorganisms can produce enzymes to use chemicals as both an energy and a carbon source, or as a means of detoxification. Unlike many other transformation processes that may result in only partial alteration of the parent compound, biodegradation can lead to complete mineralization of the substrate to carbon dioxide, water, and inorganic compounds by individual or mixed microbial populations.

There are numerous species of microorganisms in soils and waters. These organisms are metabolically diverse, multiply and adapt rapidly, have a high rate of mutation, and can grow under a variety of environmental conditions, including in the presence (aerobic) or absence (anaerobic) of molecular oxygen. Because of these characteristics, it is important to study the biodegradability of chemicals in the environments in which they are disposed of.

Photolysis may also be important in the transformation of chemicals in the environment. Photochemical transformations of chemicals in aquatic environments originate from two general types of processes--direct and indirect photolysis. Direct photolysis of a chemical occurs when the chemical absorbs the incident light and reacts; the product-formation efficiency (in moles) per einstein of light absorbed is called the reaction quantum yield. Although the chemical is photolyzed "directly", the products and reaction rates vary with oxygen and other factors in the environment that affect the lifetime or reactions of the chemical in the excited state. Indirect photolysis occurs when substances other than the chemical absorb the light energy and then either (1) transfer the excitation energy to the chemical, or (2) generate intermediates that react with the chemical. A sunlight photolysis experiment with the chemical in a natural water is useful for determining whether indirect photolysis occurs. Although there is considerable evidence that indirect photolysis is important in environmental chemistry, the mechanisms responsible for the chemical reactions usually have not been determined unambiguously. Since the mechanism may vary with the substances in the natural waters, and the quantum yield for each will be unique, a reliable and general indirect photolysis rate is difficult to estimate.

Photolysis of a chemical in the aquatic environment may be slowed by natural substances that absorb light but do not lead to photolysis, and thereby reduce the amount of light available for photolysis. Also,

the observed photoreaction of a chemical (that is, photooxidation, photoisomerization, photohydrolysis, etc.,) may occur by both direct and indirect photolysis.

Both photolysis and biodegradation affect the persistence of chemicals in the environment. This study was undertaken to estimate the effect of these processes on the persistence of DIMP and DCPD at RMA.

III OBJECTIVES

The objectives of this research were:

(1) To determine if DCPD and DIMP undergo biological transformation in the soil and water at RMA and to estimate the rates of transformation.

(2) To investigate the potential for photochemical transformations of DCPD and DIMP and to estimate the rates of transformation.

It soon became evident that volatilization was important in the environmental fate of DCPD. Therefore, this transport process was also studied, and the volatilization rate was estimated. To support these studies, analytical methods were developed to monitor the parent compounds and their potential transformation products in biological and photochemical studies and in the natural soils and waters of RMA.

Since the approaches to studying the fate processes for each chemical were different, this report contains a separate section for each chemical. This organization minimizes any confusion that might result from the conclusions reached for each compound.

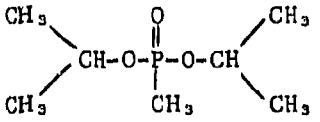
The following sections describe the experimental design, analytical methods, and results obtained from laboratory studies to determine the persistence of DCPD and DIMP in the face of microbiological and photochemical transformation processes.

IV DIISOPROPYL METHYLPHOSPHONATE (DIMP)

A. Introduction

DIMP is a by-product of nerve gas manufacture and has entered the environment by land disposal. The structure of DIMP is shown below.

CAS No. 1445-75-6



DIMP is a liquid (d = 0.976) at room temperature and has a boiling point of 174°C. Its aqueous solubility exceeds 1 g/liter.

There is little available literature on environmental processes for DIMP. Bel'skii et al's[1] hydrolysis rate data for elevated temperatures, extrapolated to 10°C, indicate a hydrolysis half-life of 530 years.

Since no literature on photochemical or microbiological transformations was found, these studies were undertaken to estimate the importance of these processes in determining the environmental fate of DIMP.

B. Materials and Methods

1. Chemicals

DIMP, isopropyl methylphosphonate (IMP), and methylphosphonic acid (MP) were obtained from Dr. William Dennis, USAMBRDL, Fort Detrick, Frederick, Maryland. ^{14}C[methyl]-DIMP was obtained from KOR Isotopes, Cambridge, Massachusetts; it had a specific activity of 8.2 mCi/mmol.

2. Water and Soil Samples

Water and sediment samples were obtained from North Bog, RMA, during August 1978 (26°C) and January 1979 (from under the ice) in sterile glass bottles. The pH of these water samples was 7.9 and 7.7,

respectively, and the dissolved oxygen content was 100% and 20% of saturation, respectively. Local waters were obtained from a pond near Searsville Lake in Woodside, California, and from a sewage aeration effluent from Palo Alto Sewage Plant.

Soil samples were obtained from RMA Pit #1 (Site 36017) and Pit #4 (Site 36017) when the North Bog water samples were collected. Mixtures of surface to 1.2-m (4-ft) deep soils were collected in sterile plastic bags. The RMA samples were shipped by air to SRI, where they were placed in refrigerated storage within 24 hours from sampling. The soils were sieved through a 2-mm screen sieve, and equal amounts of Pit #1 and Pit #4 soils were mixed for the biodegradation studies. The soil was sandy loam of pH 7.5, the field capacity was 19.3, and the organic matter content was 0.7%.

3. Analytical Methods

a. DIMP

Water and soil samples were extracted with 3 volumes of ethyl acetate, separated or filtered, dried over anhydrous Na_2SO_4, and concentrated to a few milliliters using rotary evaporation. The concentrates were analyzed by glass-capillary gas chromatography (GC) using alkali-flame ionization detection (AFID) under the following conditions.

Instrument:	Varian 2700 Gas Chromatograph modified for capillary columns
Column:	50-m SP2100 glass capillary column
Temperature:	110° isothermal
Flow rate:	0.5 ml/min N_2
Detector:	AFID
Retention time:	9.82 min, DIMP
	11.52 min, TEP (internal standard)

Quantitation was achieved by the internal standard method using triethyl phosphate (TEP) as the internal standard. Peak areas and chromatograms were recorded with a Hewlett-Packard Model 3380A Integrator-Recorder.

b. IMP, MP, and PO_4^{-3}

The methylated derivatives of IMP, MP, and phosphate (PO_4^{-3}) were also analyzed by capillary gas chromatography, but a more complex extraction, clean-up, and derivatization procedure was necessary because these materials were complexed in RMA soils and waters and not amenable to the proecdures used for DIMP.

Extraction. Approximately 20 g of soil was added to a 100-ml round-bottom flask. Then 30 ml of water was added, and a condenser, a heating mantle, and a stirring bar were attached. The soil and water were refluxed for 5 min and cooled. Then the water was decanted into a 50-ml centrifuge tube and centrifuged at 2000 rpm for 15 min. The water was decanted into a micron filter apparatus under vacuum and filtered. A 20-ml aliquot of this filtrate was set aside for column clean-up.

Column Clean-up. A small piece of glass wool was inserted into a 22-mm ID separatory column, and 38 mm of CG-120 cation resin (100-200 mesh, Na^+ form) was added. This apparatus was back-flushed with high-purity water, drained, then rinsed first with 80 ml of 50/30 water/concentrated HCl then with 60 ml of 30/30 water/concentrated HCl. The column was then rinsed with 200 ml of water to return it to pH 7. A 20-ml aliquot of the water sample was added, and the eluent was collected in a 100-ml round-bottom flask. Then 50 ml of high-purity water was added and collected in the same flask. About 1.0 ml of concentrated HCl was added to the sample, and the sample was frozen in dry ice-acetone, then lyophilized for 20 hr at about 0.5 mm Hg.

Derivitization. To the lyophilized flask, diazomethane in Et_2O/MeOH (90/10) was added until a yellow color persisted, and the flask was allowed to stand for 1 hr. Then 10 µl of triethyl phosphate (TEP, 0.572 µg/µl) was added as an internal standard, and the contents of the flask transferred to a small Teflon-lined vial (about 4 ml).

Gas Chromatography. The GC was performed under the following conditions, using the Varian 2700 GC.

Column:	50-m SP2100 glass capillary column	
Temperature:	110°C isothermal	
Flow rate:	0.5 ml/min N_2	
Detector:	AFID	
Retention times:	MP	5.99 min
	PO_4	6.53 min
	IMP	7.49 min
	DIMP	9.82 min
	TEP	11.52 min (internal standard)

Using this procedure, the recovery from spiked SRI soils and water was 105 ± 4% for MP and 104 ± 4% for IMP. Direct ethyl acetate extraction of soil gave 100 ± 2% recovery of DIMP. The same procedure was used for water samples, starting with the decantation of the water into a micron-filter apparatus.

4. UV-Visible Spectra

The UV-visible absorption spectrum of DIMP was measured on a Cary 15 UV-visible recording spectrophotometer. The spectrum above 350 nm was determined in 10-cm quartz cells; 1-cm cells were adequate for measurements below 350 nm.

5. Laboratory Photolyses

DIMP was photolyzed using a merry-go-round reactor (MGRR). The irradiation source was a Hanovia, 450-W, medium pressure Hg lamp in a borosilicate immersion well, which effectively blocks all light below 290 nm. The distance between the irradiation source and sample tubes was about 5 cm. At intervals, the sample tubes were removed from the MGRR and analyzed.

6. Development of Acclimated Culture

Water samples were mixed with a 5% volume of sediment and placed in large sterile reservoir bottles, and the sediment was allowed to settle by gravity. The supernatants were siphoned and filtered through fine-mesh polyester cloth to remove insects and unsettled particles. Biodegradation screening tests were conducted in aerated bottles and in static bottles. For the aerated bottles, 3 liters of the water sample were added to a sterile 9-liter glass bottle containing 1 liter of a solution with 4 g of phosphate buffer or Tris buffer (pH 7.5) and 2 g of $(NH_4)_2SO_4$. The bottle was fitted for sterile aeration, air exhaust, sampling, and addition of other nutrients. Concentrated aqueous solutions of DIMP were added until the desired concentration in the waters was achieved. The bottles were aerated gently at 25°C in a constant temperature room. For the static bottles, 2 liters of a water sample plus 30 ml of buffer and $(NH_4)_2SO_4$ solution [final concentration of buffer, 1 g/liter; of $(NH_4)_2SO_4$, 0.5 g/liter] were placed in a 4-liter bottle. On some occasions 1 liter of water in 2-liter bottles was used. The bottle for the DIMP biodegradation test always was closed with a cotton plug. The bottles were shaken in the beginning of the experiment, 3 times a week, and before every sampling. They were incubated at 25° and 10°C. Samples withdrawn from the bottles periodically were extracted with equal volumes of ethyl acetate (3 times) containing triethyl phosphate as the internal standard. The extracted solvents were dried with Na_2SO_4 and used for GC.

The cultures were transferred in flasks containing the test chemical and basal-salts media. The phosphate-buffer basal-salts medium contained, per liter: 1.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.5 g of

$(Na_4)_2SO_4$, 0.1 g of NaCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCl_2 \cdot 2H_2O$, 0.005 g of $FeSO_4 \cdot 7H_2O$, and 1 ml of trace elements solution; each liter of trace elements solution contained 0.1 g of H_3BO_3, 0.05 g each of $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, Na_2MoO_4, and $CoCl_2 \cdot 6H_2O$. The Tris-buffer basal-salts medium was similar, but contained 2 g of pH 7.5 Tris buffer and 0.2 g of KCl in place of the potassium phosphate salts.

7. Soil Biodegradation

Soil biodegradation studies were conducted in soil biometer flasks,[2] which are rubber-stopper-sealed 250-ml Erlenmeyer flasks with a side arm for trapping CO_2. Fifty grams dry weight of soil sample was placed in the flask, and radiolabeled and nonlabeled test chemical was added to the soil in 1 ml of distilled water. The soil and chemical were mixed well with a glass rod, and sterile distilled water was added to 80% of field capacity. To trap CO_2, 10 ml of 0.5N KOH solution was placed in the side arm. The alkaline solution was replaced with fresh solution weekly.

The following procedure was used to determine $^{14}CO_2$ evolution. One ml of KOH solution was used with 10 ml of Scintisol scintillation liquid (Isolat, Akron, OH), and radioactivity was counted. Because some DIMP was volatilized and trapped in the KOH solution, 5 ml of KOH solution was mixed with 5 ml of 10% $BaCl_2 \cdot H_2O$ solution, and the resulting solution was centrifuged or membrane-filtered, then washed twice with 5 ml of distilled water and once with 5 ml of 95% ethanol. The $BaCO_3$ precipitate was placed in a Warburg flask with 0.5 ml of water, the flask was sealed, and the precipitate was acidified with 0.3 ml of 3N HCl solution introduced through the side arm. The CO_2 was retrapped in alkaline solution in the center well, and the radioactivity was counted. In some cases 1 ml of the original KOH solution was placed in the scintillation vial, acidified with HCl, and the radioactivity as $^{14}CO_2$ counted. The difference in counts before and after acidification of the CO_2 trap was used to calculate $^{14}CO_2$.

C. Results and Discussion

1. Photolysis

The absorption spectrum from 280 to 700 nm showed that DIMP absorbs light weakly in the solar spectral region. The molar absorptivities for DIMP in this spectral region appear in Table 1.

Table 1

UV-VISIBLE ABSORPTION OF DIMP IN HEXANE

λ	ε DIMP (0.0165M)
230	0.333
300	0.222
320	0.192
340	0.162
360	0.121
380	0.106
400	0.106 [a]

[a] Absorption tailed out to 600 nm, we believe because of instrumental or cell problems; the values listed are probably high.

The results of attempts to photolyze DIMP at > 290 nm in both distilled and RMA waters showed no loss of parent compound, within an analytical error of ± 2%, after 232-hr reaction time, Table 2. The stability of DIMP to direct and indirect photolysis shows that photolysis is not important in determining the environmental fate of DIMP.

Table 2

RESULTS OF PHOTOLYSIS OF DIMP IN WATERS AT > 290 NM

Water	ΔT (hr)	DIMP Remaining (%)[a]
Distilled	89	100
North Bog-shallow	89	100
Distilled	232	100
North Bog-shallow	232	100

[a] Blank control maintained in dark gave 100% recovery

2. Biodegradation

Development of Acclimated Culture. The microorganisms in North Bog water and in RMA soil were grown in basal-salts medium with glucose (1 g/liter) and Difco yeast extract (0.1 g/liter). Then they were grown in shaker flasks with different concentrations of DIMP up to 100 ppm in basal-salts, glucose, and yeast-extract media. Comparisons of broth turbidity showed no growth inhibition, which indicated that a 100-ppm level of DIMP could be used to acclimate cultures.

The acclimation of DIMP biodegradation organisms with North Bog water collected in summer began in aerated 9-liter bottles with 10- and 3-ppm levels of DIMP, Tris buffer (pH 7.5), and $(NH_4)_2SO_4$. Autoclaved water with 10-ppm DIMP was used as a sterile control. The bottles were incubated at 25°C. Static bottles without buffer and ammonium salts were also incubated with DIMP (10 ppm) at 25° and 10°C. Analysis showed that DIMP was not biodegraded detectably during 12 weeks of incubation.

During this screening test, aliquots of water samples were inoculated into flasks containing Tris-buffer basal-salts medium with and without glucose (100 ppm): glucose (100 ppm) and yeast extract (20 ppm); glucose (100 ppm) and glycerol (100 ppm); or glucose, glycerol, and succinate (100 ppm each) as extra carbon sources. None of the flasks showed DIMP biodegradation.

The waters from the Palo Alto sewage plant aeration tank and from the pond near Searsville Lake, Woodside, California, were also tested in static bottles for the DIMP biodegradability with and without glucose, and with and without glycerol plus succinate. No DIMP biodegradation was observed.

To investigate the biodegradabilities of MP and IMP, the North Bog water in the 9-liter aerated bottles and Palo Alto aeration tank effluent water were inoculated into the flasks containing Tris-buffer basal-salts medium, glucose, glycerol, and succinate, with or without MP or IMP. The turbidity of the broth after 2 to 3 days in the flasks containing sugar and MP or IMP showed microbial growth, while that in the flasks containing only sugars (no phosphonate) showed very low turbidity. This result indicates that the microbes in North Bog water and in Palo Alto water could readily use MP or IMP as a phosphate source and split the carbon-phosphorus linkage, but could not use DIMP. When trimethylphosphate (TMP) was tested in place of MP in the above medium, it was also biodegraded.

When the North Bog water microbes were inoculated in the Tris-buffer basal-salt media with sugars and DIMP with added MP, IMP, or TMP, organisms grew with MP, IMP, or TMP as phosphate sources, but DIMP was not degraded. These compounds did not serve as cometabolic substrates or as enzyme inducers.

Isopropanol was added as a carbon source in the Tris-buffer basal-salts medium with DIMP. Although isopropanol was used by microorganisms as the sole carbon source in medium with phosphate, DIMP was not degraded. Like MP, IMP, and TMP, isopropanol did not serve as a cometabolic substrate or enzyme inducer.

North Bog water was collected again in January, and the acclimation test was repeated in static bottles. The water with and without Tris buffer, along with a sterile control, was incubated at 10°C. The water with Tris buffer was also incubated at 25°C. Again, chemical analysis showed no detectable DIMP biodegradation during 12 weeks of incubation.

Into biometer flasks containing Tris-buffer basal-salts medium, glucose, glycerol, 2 μCi of DIMP, and unlabelled DIMP (10 ppm), we inoculated North Bog water, Palo Alto water, and pond water microbes. Ten milliliters of 0.5N KOH solution was placed in the side arm. No radioactive $^{14}CO_2$ was observed in the KOH solutions during 6 weeks of incubation.

DIMP was biodegraded to CO_2 in RMA soil. To investigate whether this was caused by the presence of some nutrients in the soil, 20 g of RMA soil was extracted with 50 ml of boiling water, and 2.5 ml of the filtered supernatant was added to a 250-ml flask containing 50 ml of

Tris-buffer basal-salts medium with glucose and glycerol (200 ppm each). In another flask, 1 g of soil was added in place of the soil extract. Radiolabeled ^{14}C-DIMP (1.4 μCi) and DIMP (10 ppm) were added, and North Bog water was inoculated from the 9-liter bottle. The flask was closed with a rubber stopper equipped with a Teflon-lined screw-capped test tube, which has a hole under the rubber stopper so KOH solution can be placed in the tube to trap CO_2.[3] No evolution of $^{14}CO_2$ was found in these flasks, indicating that extra nutrients in the soil were not helping aqueous DIMP biodegradation.

Soil from the 13-week-old preliminary DIMP soil biodegradation flask, which contained acclimated microorganisms, was also inoculated into the above medium. No $^{14}CO_2$ evolution from methyl ^{14}C-DIMP was observed during 6 weeks of incubation. Thus, it appeared that environmental factors, not the microorganisms, are responsible for the persistence of DIMP in water.

The soil percolator of Goswami and Green[4] was used to acclimate the biodegradation culture from RMA soil. We then percolated 500 ml of an aqueous solution of DIMP (20 ppm) in Tris-buffer basal-salts medium with glucose and glycerol continuously through 50 g of RMA soil column. After 32 days 87% of the added DIMP still remained in the medium. This reduction may have been caused by adsorption, not by biodegradation. No further decrease was observed for 18 weeks.

In a parallel experiment, at 21 days the aqueous broth was inoculated into Tris-buffer basal-salts medium containing glucose, glycerol, and DIMP, IMP, or MP. Good growth was observed for media with MP or IMP, but no growth was observed in the medium with DIMP. After 11 days in DIMP medium, no significant reduction of DIMP concentration was noted.

Organisms reported to degrade IMP or other phosphonates were obtained from Dr. C. G. Daughton of M. Alexander's laboratory at Cornell University. The organisms--Pseudomonas testosteroni and strain No. 12[5,6] --were tested with Tris-buffer basal-salts medium and sugars with DIMP, IMP, and MP. They readily used MP and IMP as the sole phosphate source, but could not use DIMP.

Biodegradation Rate in Water. Since no biodegradation of DIMP in water samples was observed and no acclimated culture system developed, aquatic biodegradation studies were not conducted.

Soil Biodegradation. Preliminary soil biodegradation studies were begun in biometer flasks with RMA soil collected in August. The soil was a mixture of soils from Pit #1, Pit #4, and the North Bog. Radioactive methyl-^{14}C-DIMP (2.28 μCi in 1 ml of H_2O) was mixed into

50 g of the soil mixture, which originally contained 2.7 ppm DIMP. Sampled weekly, the KOH solution in the side arm was found to be trapping ^{14}C from volatilized DIMP and $^{14}CO_2$. The biometric flask containing sterile soil and ^{14}C-DIMP also had radioactivity in the KOH solution in the side arm.

The KOH solution from the flask with nonsterile soil was extracted with ethyl acetate, and the concentrated extract was spotted on a silica gel TLC plate and developed with 10% isopropanol/CH_2Cl_2 solvent. X-ray film radioautography showed that radioactivity in the extract was mainly from DIMP; no volatile metabolites were detected. The $^{14}CO_2$ in the KOH solution had to be precipitated with $BaCl_2$, the washed $BaCO_3$ acidified, and the CO_2 retrapped to make the $^{14}CO_2$ count. The $^{14}CO_2$ radioactivity was calculated as the percentage of ^{14}C originally added to the soil. The results are shown in Figure 1.

Figure 1 showed that DIMP was degraded to CO_2 by RMA soil microorganisms, but that biodegradation was very slow, with only 13.4% of the original activity being evolved as $^{14}CO_2$ after 34 weeks of incubation. Total ^{14}C removed in KOH solution during this period was 32% of the original ^{14}C. At this rate, it will take a year for the accumulated total ^{14}C to reach 20% and more than 2 years to achieve 50% evolution as $^{14}CO_2$.

We also tested a soil from SRI International grounds for DIMP degradation. The pattern of $^{14}CO_2$ evolution observed in SRI soil was similar to that in the RMA soil. To test whether the reductive generation of methane was possible, evolved gases were bubbled through a dodecane trap, which then was analyzed for methane by gas chromatography/mass spectrometry (GC/MS). Methane was not detected, indicating that the production rate was very low or that this mode of transformation is not occurring.

After soil samples were collected in January, the ultimate soil biodegradation testing was initiated with duplicate flasks at two temperature levels and at two DIMP levels. Soil collected in the summer and air dried was used for the 25°C incubation test, and soil collected in the winter was used for the 10°C incubation test. The soils collected in August and January had 2.9-ppm and 1.0-ppm levels of DIMP, respectively. Radiolabeled ^{14}C-DIMP (1.4 μCi) was added to the soils, with and without extra unlabeled DIMP (10 ppm).

Another flask containing additional DIMP (10 ppm) and ^{14}C-DIMP was inoculated with 1 ml of broth from the 8-week-old soil percolator at the start. It was again inoculated with 2 g of soil from the 22-week-old preliminary DIMP biodegradation test soil biometer flask after 4 weeks of incubation because the soil percolator did not acclimate the biodegradation organisms.

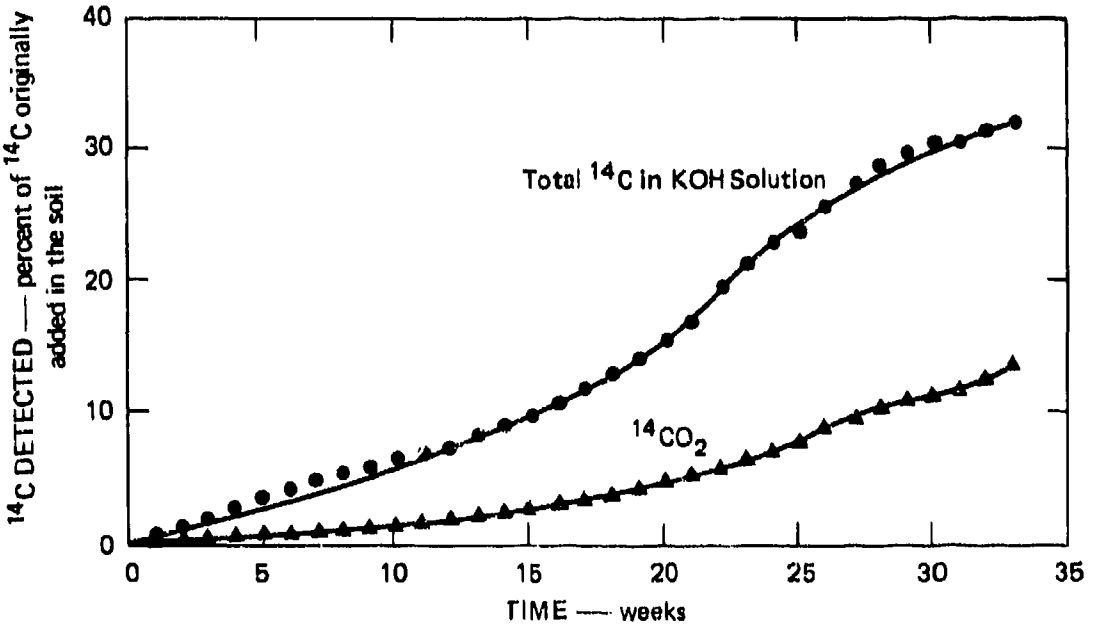


FIGURE 1 ACCUMULATED $^{14}CO_2$ AND TOTAL ^{14}C COUNT IN PRELIMINARY TEST FOR DIMP SOIL BIODEGRADATION

The $^{14}CO_2$ evolution during 17 weeks of incubation is shown in Figure 2. At 25°C, the percent of accumulated $^{14}CO_2$ from both the 2.9-ppm flasks and the 12.9-ppm flasks were about the same. The CO_2 production rates are therefore not a function of DIMP concentration. When soil was inoculated with the acclimated soil, the $^{14}CO_2$ evolution rate increased, and reached over 5% at 17 weeks; the soils without inoculation produced about 1.5%. At this rate, it will take more than 3 years to reach 50% mineralization in a noninoculated soil and more than 1 year in an inoculated soil.

The DIMP soil incubated at 10°C did not produce significant amounts of $^{14}CO_2$ (less than 0.1%). DIMP biodegradation in soil was almost completely halted at this temperature, and the evaporation loss was lower. The accumulated total ^{14}C counts in the KOH solution were about 15% of the added ^{14}C at 25°C and 3% at 10°C; it was 20% in the flask with acclimated soil at 25°C during 17 weeks of incubation.

Analytical Chemistry. The RMA soils and waters were analyzed for DIMP and its two primary hydrolysis products, IMP and MP, as well as phosphate ion, using capillary GC for all species and confirming phosphate by colorimetry.[7] A summary of results appears in Table 3. Components were identified by capillary gas chromatographic retention time and confirmed by GC/MS. A typical gas chromatographic profile of a sample extract appears in Figure 3.

Chemical Hydrolysis. The appearance of IMP and MP in RMA water suggested that catalytic hydrolysis could be occurring. To test this possibility, 100 ml of North Bog water containing 0.26 ppm of DIMP was refluxed for 1 hr and reanalyzed for DIMP. The recovery of DIMP was 98% of the original concentration, indicating that no catalytic processes are transforming DIMP in the environment.

D. Conclusions

Data for the photolysis of DIMP in distilled water and in a natural water sample showed no loss after 232 hr of photolysis with the Hg lamp filtered to exclude all wavelengths below 290 nm. Direct or indirect photolysis therefore is not important in aquatic systems.

Biodegradation of DIMP was not observed in natural waters incubated for 12 weeks or in aqueous medium incubated with acclimated soil microorganisms for 6 weeks. Therefore, we conclude that this transformation process also is not important in aquatic systems.

The methyl carbon in DIMP was observed to biodegrade to CO_2 very slowly at 25°C in soil, and almost no biodegradation was observed at 10°C. Thus, temperature variations will be important in estimating the persistence of DIMP in an environment such as that of RMA, and a half-life in excess of two years is predicted.

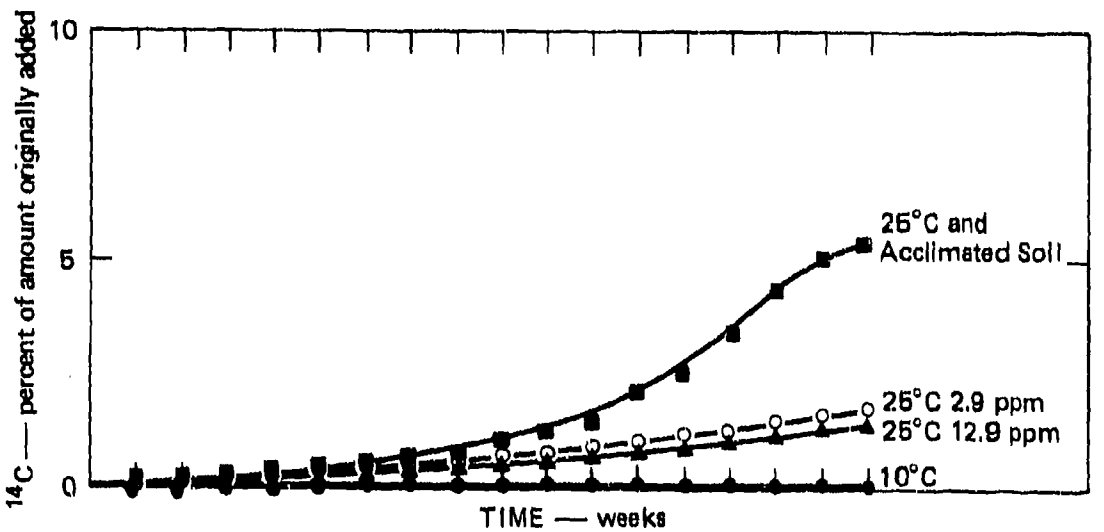


FIGURE 2 ACCUMULATED $^{14}CO_2$ EVOLUTION IN SOIL DIMP BIODEGRADATION TESTS AT 25°C AND 10°C

Table 3

SUMMARY OF RESULTS FOR ANALYSES OF DIMP, IMP, MP, AND PO_4^{-3} IN RMA WATERS AND SOILS

Sample	Collection Date	Concentration (ppm) DIMP	IMP	MP	PO_4^{-3}
North Bog surface	8/78	0.25	0.012	0.252	1.55 (1.15)[a]
North Bog deep	8/78	0.26	0.026	0.118	2.04 (1.90)[a]
North Bog south	1/79	0.27	0.037	0.106	1.40 (1.10)[a]
North Bog north	1/79	0.26	0.018	0.136	1.23 (1.05)[a]
Pit #4 ground water	1/79	1.10	0.100	0.068	0.025 (0.031)[a]
Pit #1 soil	8/78	0.11	0.016	0.21	2.20
Pit #1 soil	1/79	0.10	0.008	0.46	0.50
Pit #4 soil	8/78	9.10	0.012	2.00	0.78
Pit #4 soil	1/79	1.71	0.058	5.30	1.50
Lake F soil	8/79	0.54	1.20	27.70	29.8
North Bog soil	8/79	0.050	0.012	0.43	1.20

[a] Colorimetric value.

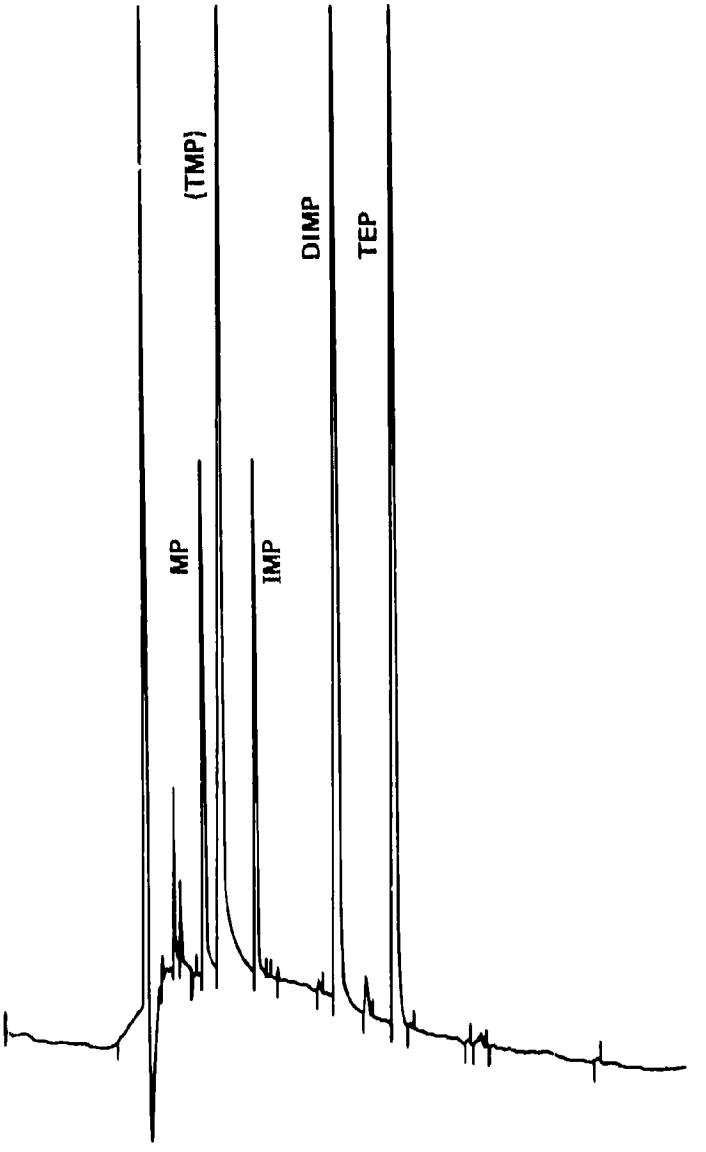
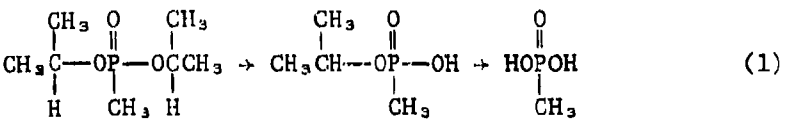


FIGURE 3 GAS CHROMATOGRAPHIC PROFILE OF OF DERIVATIZED NORTH BOG WATER

Certain microorganisms can use a variety of organophosphonic acids as a source of phosphorus,[8-12] and one microbial phosphonatase was studied and described.[13] The microorganisms isolated from soil and sewage can use aryl and alkyl phosphonate, including MP and IMP,[5,14] but no biodegradation of DIMP was reported.

We were able to grow microorganisms using MP or IMP as the sole phosphorus source in a phosphate-deficient organic compound supplement medium for North Bog water, Palo Alto sewage aeration tank water, Woodside, CA pond water, RMA soil, or SRI soil. It seems, therefore, that MP- and IMP-using microbes are widely present in natural environment, and that hydrolysis of DIMP to IMP is the rate-limiting step (Eq. 1) in determining the environmental persistence of DIMP.



(1)

When inorganic phosphate is present in growth media, microorganisms exhibit diauxic use of MP and inorganic phosphate. The use of MP was suppressed in the presence of inorganic phosphate.[6] This could be why we still can find MP and IMP in the North Bog water--because substantial concentrations of inorganic phosphate are present in the water. Many soils have a high capacity to bind inorganic phosphate and a low capacity to bind ionic alkyl phosphorous compounds. As a result, the diauxic suppression of phosphonate by inorganic phosphate is abolished by phosphate-binding soil.[6] Therefore, the DIMP biodegradation soil microbes may have some advantage over microorganisms in aquatic environments due to limited access to phosphorus.

In spite of its high solubility in water and relatively high boiling point, DIMP evaporates from soils and to a lesser extent from waters. The ^{14}C-DIMP was detected in the $^{14}CO_2$ traps and $^{14}CO_2$ had to be separated by $BaCO_3$ precipitation. The DIMP loss from the soil by evaporation may be faster than the loss of the labeled methyl carbon of DIMP in the ultimate biodegradation. In these ultimate soil degradation studies, it may take the $^{14}CO_2$ evolution more than 2 years to account for 50% of the DIMP decomposition. Total ^{14}C trapped in KOH solution may take 1 year to reach 50% at 25°C which may include volatilization losses. At winter temperatures, the rate will be even slower.

Although IMP and MP are found in significant concentrations in RMA soils and water, from our studies we conclude that they do not arise from photolytic or microbial transformation processes in the environment. Chemical hydrolysis also should not be significant, according

to Bel'skii's data, from which a half-life of 530 years can be estimated for hydrolysis. The data suggest that the IMP and MP in the RMA environment are residues from chemical manufacturing disposed of on land.

E. Recommendations

To further elucidate the environmental fate of DIMP, we recommend that a rate constant for volatilization in water be determined experimentally. Our microbiological studies show that this process is important and will proceed more rapidly than any biological or photolytic transformation process.

We also recommend that the sediment partition coefficient of DIMP be determined. We do not expect this value to be large (based on DIMP's aqueous solubility), but it should be determined for an environmental fate data base.

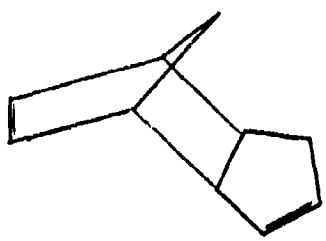
V DICYCLOPENTADIENE (DCPD)

A. Introduction

DCPD is a common precursor in manufacturing pesticides (e.g., dieldrin, chlordane). Its presence in the environment is caused by the land disposal of pesticide wastes.

DCPD is a waxy solid and has a melting point of 32°C. The structure of DCPD is shown below. Although DCPD can exist in cis- and trans-forms, the trans-form is usually prevalent.

CAS No. 0077-73-6



The aqueous solubility of DCPD has not been determined exactly. Although DCPD is considered insoluble in water, we have prepared concentrations greater than 50 ppm in our laboratory. It is estimated that solubility in water would not exceed 100 ppm.

No literature data on environmental transport and transformation processes of DCPD were found. This study was undertaken to provide information on the potential for photochemical and microbial transformations and to estimate rates of transformation as they might occur in the environment.

B. Materials and Methods

1. Chemicals

DCPD obtained from Aldrich Chemical Company (Milwaukee, Wisconsin) was determined to be 94% pure by GC. The purity was increased to 98% by distillation under vacuum. Distilled samples were stored at -20°C until used.

DCPD uniformly labeled with ^{14}C, was obtained from KOR Isotopes (Cambridge, Massachusetts), possessed a specific activity of 9.9 mCi/mmol. Stock solutions of ^{14}C-DCPD were prepared in acetone.

2. Water and Soil Samples

Water and sediment samples were obtained from North Bog, RMA, during August 1978 (25°C) and January 1979 (from under the ice) in sterile glass bottles. The pHs of the water samples were 7.9 and 7.7, respectively, and the dissolved oxygen content was 100% and 20% of saturation, respectively. Local waters were obtained from a pond near Searsville Lake in Woodside, California and a sewage aeration effluent from Palo Alto sewage plant.

Soil samples were obtained from RMA Pit #1 (Site 36017) and Pit #4 (Site 36014) when the North Bog waters were collected. Mixtures of surface to 1.2-m (4-ft) deep soils were collected in sterile plastic bags. The RMA samples were shipped by air to SRI, where they were placed in refrigerated storage less than 24 hours from the sampling time. The soils were sieved through 2-mm screen sieves, and equal amounts of Pit #1 and Pit #4 soils were mixed for the biodegradation studies. The soil was sandy loam, had a pH of 7.5, field capacity of 19.3%, and organic matter content of 0.7%.

3. Development of Acclimated Culture

Water samples mixed with sediment (about 5% volume) were placed in a large sterile reservoir bottle, and the sediment was allowed to settle by gravity. The supernatants were siphoned and filtered through fine-mesh polyester cloth to remove insects and unsettled particles. Biodegradation screening tests were conducted in aerated or in static bottles. For the aerated bottles, 3 liters of the water sample was added to a sterile 9-liter glass bottle containing 1 liter of a solution with 4 g of phosphate buffer or Tris buffer (pH 7.5) and 2 g of $(NH_4)_2SO_4$. The bottle was fitted for sterile aeration, air exhaust, sampling, and addition of other nutrients. Concentrated DCPD in dimethylsulfoxide (40 mg/ml) was added until the desired concentrations in the waters were reached. The bottles were aerated gently at 25°C in a constant temperature room. For the static bottle tests, 2 liters of a water sample and 30 ml of buffer and $(NH_4)_2SO_4$ solution [final concentration of buffer, 1 g/liter; of $(NH_4)_2SO_4$, 0.5 g/liter] was placed in a 4-liter bottle. On some occasions, 1 liter of water and 2-liter bottles were used. Bottles of water containing DCPD were closed with glass stoppers to inhibit volatilization. The bottles were shaken in the beginning of the experiment, 3 times a week, and before every sampling. They were incubated at 25 and 10°C. Samples were

withdrawn from the bottles periodically and extracted with ethyl acetate for chemical analysis. The DCPD water was extracted 2 times with one-quarter volume of ethyl acetate containing naphthalene as the internal standard for GC.

The cultures were transferred in flasks containing test chemical and basal-salts media. The phosphate-buffer basal-salts medium contained, per liter: 1.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.5 g of $(NH_4)_2SO_4$, 0.1 g of NaCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCl_2 \cdot 2H_2O$, 0.005 g of $FeSO_4 \cdot 7H_2O$, and 1 ml of trace elements solution. The trace elements solution contained, per liter, 0.1 g of H_3BO_3, 0.05 g each of $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, Na_2MoO_4, and $CoCl_2 \cdot 6H_2O$. The Tris-buffer basal-salts medium was similar, but contained 2 g of pH 7.5 Tris buffer and 0.2 g of KI in place of the potassium phosphate salts.

4. Soil Biodegradation

Soil biodegradation studies were conducted in soil biometer flasks,[2] which are rubber-stopper-sealed 250-ml Erlenmeyer flasks with a side arm for a CO_2 trap. Fifty grams (dry weight) of the soil sample was placed in the flask. Radiolabeled and unlabeled DCPD was added to the soil with 1 ml of acetone. The soil and chemical were mixed well with a glass rod to allow the acetone to evaporate, and sterile distilled water was added to 80% of field capacity. To trap CO_2, 10 ml of 0.5N KOH solution was placed in the side arm. The alkaline solution was replaced weekly with fresh solution.

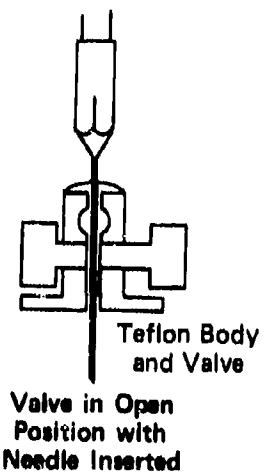
The following procedure was used to determine the amount of evolved $^{14}CO_2$. One ml of KOH solution was used with 10 ml of Scintisol scintillation liquid (Isolab, Akron, OH), and radioactivity was counted. Because some DCPD volatilized and was trapped in the KOH solution, 5 ml of KOH solution was mixed with 5 ml of 10% $BaCl_2 \cdot H_2O$ solution, centrifuged or membrane-filtered, and washed twice with 5 ml of distilled water and once with 5 ml of 95% ethanol. The $BaCO_3$ precipitate was placed in a Warburg flask with 0.5 ml of water, the flask was sealed, and the precipitate was acidified with 0.3 ml of 3N HCl solution from the side arm. The CO_2 was retrapped in alkaline solution in the center well, and the radioactivity was counted. The difference in counts before and after acidification of the CO_2 trap solution was used to calculate $^{14}CO_2$.

5. UV-Visible Spectra

The UV-visible absorption spectrum of DCPD was measured on a Cary 15 UV-visible recording spectrophotometer. The spectrum above 350 nm was determined in 10-cm quartz cells; 1-cm cells were adequate for measurements below 350 nm.

6. Laboratory Photolysis

Initial studies with DCPD solutions were complicated by DCPD losses during analysis of photolyzed solutions and controls. This may have been because the DCPD volatilized into the ullage space in the 10-cm photolysis tubes, then escaped when the screw cap on the tube was removed for sampling with a syringe. These problems were resolved by using shorter tubes that were filled to displace all air in the tube, and using Minnert screw caps (Pierce Chemical); these Teflon caps were fitted with a silicone septum for sampling with a syringe and with a valve for sealing the solution in the tube (see Figure 4).



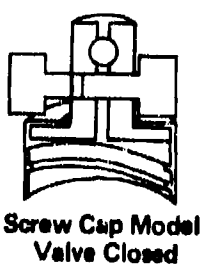


FIGURE 4

Figure 4 VALVE FOR SAMPLING DCPD SOLUTIONS DURING PHOTOLYSIS STUDIES

To minimize volatilization and obtain constant initial concentrations of DCPD, the following procedure was adopted:

A solution containing 2 mg of DCPD per milliliter of acetonitrile was prepared in a volumetric flask. A 50-µl aliquot of this solution was then transferred by syringe into a 50-ml volumetric flask and diluted to the mark with distilled or natural water. This solution, now 2-ppm DCPD, was then taken up in a 50-ml syringe with no air space and injected into the photolysis tube through the septum in the Teflon cap, displacing all air in the photolysis tube before it was sealed off with the cap valve. This procedure provided identical DCPD solutions in each tube filled from the same syringe. Samples could then be withdrawn from the tubes with the GLPC injection syringe with good reproducibility.

Photolyses of DCPD were performed using a MGRR. The irradiation source was a Hanovia, 450-W, medium-pressure Hg lamp in a borosilicate immersion well, which effectively blocks all wavelengths below 290 nm. The distance between the irradiation source and sample tubes was about 5 cm. At intervals, the sample tubes were removed from the MGRR and analyzed.

7. Sunlight Photolyses

Sunlight photolyses of DCPD were conducted in the tubes used in the laboratory photolyses. The tubes were placed in a rack that held them at an angle of 60° from horizontal. The rack was located outside in a sunlit area free of excessive reflections from walls and windows and without morning or afternoon shadows. The rack was brought inside each night and taken outside each morning to minimize temperature fluctuations that could have caused the Teflon seal cap to leak.

8. Analytical Methods

Biological Samples. In biodegradation studies, DCPD was analyzed by extracting aqueous solutions with ethyl acetate containing known amounts of naphthalene (internal standard) followed by GC analysis under the following conditions:

Instrument:	Hewlett-Packard Model 5711 Gas Chromatograph
Column:	1.8 m (6 ft) x 2 mm glass column packed with 10% DC-200 on 80/100 mesh Gas Chrom Q
Temperature:	85°C (2 min hold) → 140°C at 4°/min
Flow rate:	25 ml/min N_2
Detection:	Flame ionization
Retention time:	339 s DCPD
	574 s naphthalene (internal standard)

RMA Waters. RMA waters were analyzed by direct aqueous injection under the following conditions:

Instrument:	Hewlett-Packard Model 5711 Gas Chromatograph
Column:	1.8 m (6 ft) x 2 mm glass column packed with 13% DC-200 on 80/100 mesh Gas Chrom Q
Temperature:	100° isothermal
Flow rate:	30 ml/min N_2
Detector:	Flame ionization
Retention time:	2.28 min

Quantitation was achieved by the external standard method.

Photolytic studies. The analysis of DCPD in photolytic experiments was performed by GC using the following conditions:

Instrument:	Varian Model 3700 Capillary Gas Chromatograph
Column:	30 m x 0.25 mm ID glass capillary column coated with FFAP
Temperature:	100° isothermal
Flow rate:	1 ml/min N_2
Detection:	Flame ionization
Retention time:	5.6 min

Quantitation was achieved by the external standard method.

In all analytical studies, peak areas were determined by digital integration using a Hewlett-Packard Model 3380A Integrator-Recorder or a Varian CSD 111 chromatography data system.

9. Volatilization Rate

The rates of DCPD loss from water and oxygen reaeration of the same solution were measured at a variety of stirring speeds. Measurements were made on a solution in a 1000-ml beaker. Stirring was provided by a three-bladed paddle driven by a variable speed motor, and stirring rates were measured with a stroboscopic tachometer. The solution was recirculated at a constant speed across the membrane of a dissolved oxygen probe by an all-teflon metering pump. The beaker was not temperature controlled, although it usually remained about 21°C.

A stock solution of DCPD (0.0529 g/ml) was prepared in methanol. For each run, 0.30 ml of this stock solution was added to approximately 850 ml of deoxygenated distilled water. (Deoxygenation was accomplished by bubbling nitrogen through the water for 15 min before adding DCPD.) After shaking, the stoppered flask containing the solution was submerged in an ultrasonic cleaning bath for 10 to 20 min, until the DCPD was dissolved. The solution was allowed to equilibrate at room temperature, then was poured gently into the beaker. The pump and stir motor were turned on, and measurements began immediately.

One-milliliter aliquots of solution were removed periodically with a syringe for DCPD analysis. Each aliquot was mixed with 1.00 ml of acetone containing naphthalene (2.98×10^{-5} g/ml) as an internal standard. The mixed solutions were analyzed on an HP-5750 gas chromatograph equipped with a 5% SE-30 column (10' x 1/8" stainless steel), a flame ionization detector, and a Spectraphysics System I integrator. The column temperature was 65°C, and the injector temperature was 140°C. Both these temperatures were low enough to avoid decomposition of the DCPD. The relative concentration of DCPD was taken as the ratio of the DCPD peak area to the naphthalene peak area. The concentration of dissolved oxygen was monitored with a Delta Scientific D.O. Meter.

C. Results and Discussion

1. Photolysis Studies

The UV-visible absorption spectrum shows that DCPD absorbs light weakly in the solar spectral region. The molar absorptivities for DCPD at various wavelengths in the solar spectral region appear in Table 4.

Data for the photolysis of 2-ppm DCPD in distilled water and in the natural water samples at higher than 290 nm are shown in Table 5. DCPD in distilled water in parallel runs showed no loss after irradiation with wavelengths above 290 nm for 110 hr, indicating that direct photolysis of DCPD is slow.

Data in Table 5 also show that DCPD does undergo indirect photolysis in two natural water samples from the North Bog. These data, plotted in Figure 5, show that the photolysis rate of DCPD slows with time. We did not determine the products of the reaction or the mechanism of the photoreaction in the natural waters. Several indirect photolysis mechanisms could explain loss of DCPD in natural waters, including singlet-oxygen reactions, photo-initiated free-radical reactions, or triplet-sensitized reactions.

Table 4

UV-VISIBLE ABSORPTION OF DCPD IN HEXANE[a]

λ	ε DCPD (0.0199M)
280	9.69
300	6.28
320	4.82
340	1.61
360	0.683
380	0.253
400	0.125[b]

[a] DCPD is too insoluble in water to obtain absorption spectra.

[b] Absorption tailed out to 600 nm, we believe because of instrumental or cell problems; the values listed are probably high.

Table 5

PHOTOLYSIS OF 2-PPM DCPD ABOVE 290 NM[a]

Photolysis Time (hours)	DCPD Remaining (%)
Distilled Water	
0	100, 100
66	102, 105 (100)[b]
110	99, 15 (104)
North Bog Deep Water	
0	100
24	83
48	74 (100)
101	65
168	60 (100)
North Bog Shallow Water	
0	100
48	81
120	59 (100)
162	47
258	44

[a] Light source: 450-W, medium-pressure Hg lamp filtered by borosilicate glass.

[b] Numbers in parentheses indicate recoveries of control samples.

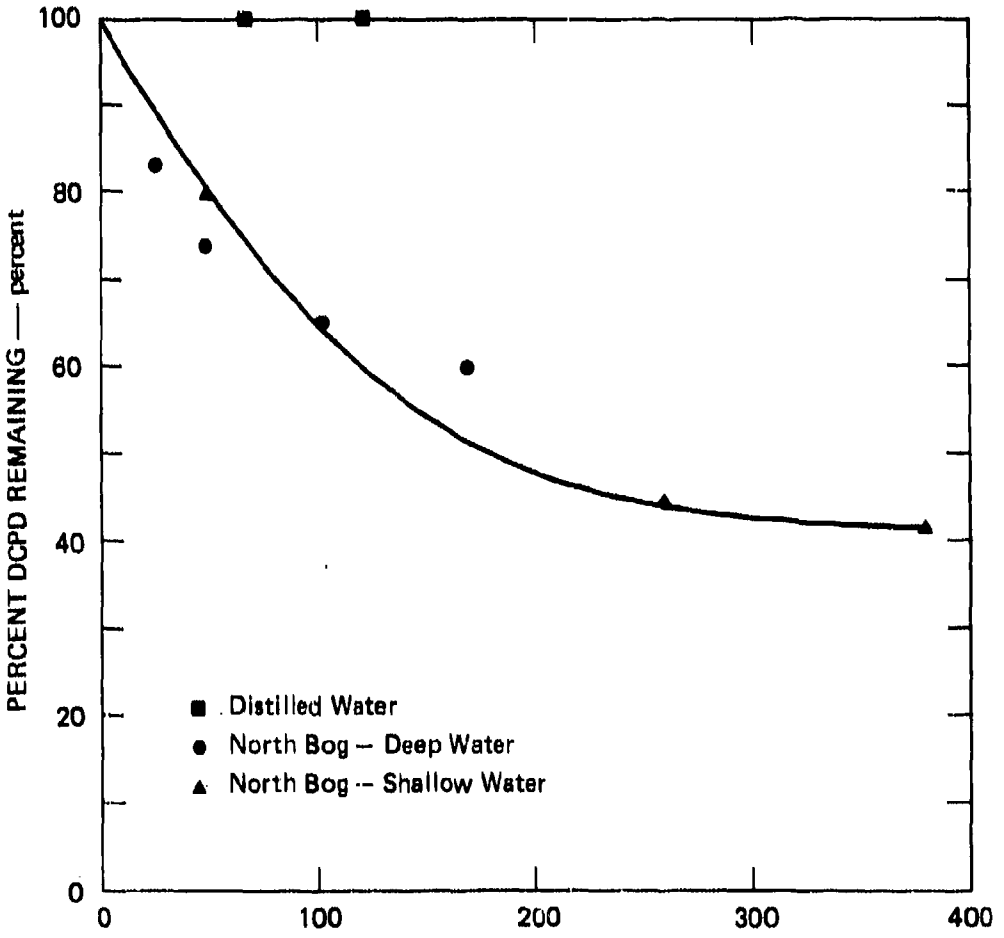


FIGURE 5 PHOTOLYSIS OF DCPD IN DISTILLED AND NATURAL WATER
(Wavelength > 290 nm)

Since substances in natural waters promote photoreaction of DCPD, further studies were performed. To study the stability of the UV-absorbing substances in natural water, a sample of North Bog water was photolyzed in the same system, at > 290 nm, as the DCPD experiments. Data at three wavelengths for the absorbance of the water before and after 70-hour photolysis are given in Table 6.

Table 6

SPECTRAL DATA FOR PHOTOLYZED DEEP NATURAL WATER

Wavelength	Absorbance	
	Before Photolysis	After Photolysis
300	0.077	0.018
400	0.026	0.001
500	0.12	$\sim$ 0

These data show that the UV-absorbing chromophores above 290 nm are destroyed by photolysis. Since DCPD does not photolyze in distilled water and the slowing of photolysis of DCPD in natural waters corresponds to the decrease in absorbance of the natural water, the indirect photolysis of DCPD must be due to chromophores associated with materials in the North Bog deep water.

Because DCPD was transformed in natural waters when irradiated with a Hg lamp filtered to provide only wavelengths above 290 nm, the photolysis experiments had to be conducted in sunlight. These experiments were necessary because our present knowledge of the indirect photolysis in aquatic environments does not enable us to estimate indirect photolysis rates from laboratory data alone. Data for sunlight photolyses of DCPD in the two natural waters (Table 7) show that no loss of DCPD from distilled or the natural waters had occurred after 80 hours of sunlight exposure. The losses shown at 130 hr are believed to have resulted from sampling and analysis problems, since the same tubes were used in the two natural water experiments, and some loss may have occurred in the longer experiment. The data for the loss of DCPD at 130 hr also do not agree with the results of the Hg lamp experiments, in which the photoreaction rate of DCPD slowed as the natural water was photolyzed. Assuming a nominal analytical experimental error of $\pm$ 7% for DCPD at 80 hr, the rate constant for photolysis of DCPD in natural waters in sunlight is greater than 9×10^{-4} hr^{-1} and the half-life is 760 hr; the 760-hr half-life corresponds to a 76-day half-life, assuming 10 hr of sunlight each day.

Table 7

SOLAR PHOTOLYSIS OF DCPD

Water	Δt (hr)[a]	DCPD Remaining (%)[b]
Distilled	80	100
	130	100
North Bog, deep	80	100
	130	75 ± 7%
North Bog, shallow	80	100
	130	85 ± 7%

[a] Total hours exposed to sunlight

[b] Tubes sampled at 130 hr had small ullage spaces above solutions: values at 130 hr should be considered approximate minimum values. One distilled water tube not previously sampled gave 100% recovery at 130 hr.

2. Biotransformation

Development of Acclimated Culture. The microorganisms in North Bog water and RMA soil grown in basal-salts medium with glucose and Difco yeast extract did not show growth inhibition when DCPD was added at 10 ppm or less; higher concentrations of DCPD did slow the growth. Therefore, the experiments were conducted using concentrations of 10 ppm or lower.

The acclimation of DCPD biodegradation organisms with North Bog water collected in summer was initiated in aerated 9-liter bottles with 10-ppm and 3-ppm levels of DCPD, pH-7.5 phosphate buffer, and $(NH_4)_2SO_4$; autoclaved water was used as the sterile control. The bottles were incubated at 25°C. Static bottles without buffer and $(NH_4)_2SO_4$ were also incubated with 10-ppm DCPD at 25° and 10°C. It soon became apparent that DCPD was very volatile in water, and DCPD was lost both from the test waters and from the control. Therefore, DCPD was added weekly, aeration was kept very low, and the water from the bottle was blindly inoculated into phosphate-buffer basal-salts medium with 10-ppm or 5-ppm DCPD, with or without yeast extract, in the Teflon-lined screw-capped or glass-stoppered flasks. DCPD analysis showed that the biodegradation could not be detected; the parallel control also lost DCPD, and often the disappearance of DCPD from the control bottles or transferred flasks was faster than in the bottles or flasks with microorganisms. Presumably, the microbes absorb part of the DCPD, and this fraction is retained in the broth.

Palo Alto sewage water was used to test the cometabolic effect of some chemicals. Media containing DCPD (5 ppm) and cyclopentene, cyclopentane, cyclopentanol, cyclohexene, cyclohexane, isopentane, or naphthalene (10 ppm) were inoculated with the sewage aeration tank water microorganisms. No chemical tested helped increase the rate of DCPD disappearance.

The North Bog water was collected again in January, and the acclimation test was repeated with 5-ppm DCPD in static bottles containing glass stoppers. The water, with and without phosphate buffer, along with the sterile control, were incubated at 10°C. The water with buffer also was incubated at 25°C. Again chemical analysis showed DCPD disappearance from the water samples and from the sterile control. These results were not sufficient to indicate that biodegradation was occurring.

The organisms from the North Bog test bottle, Palo Alto sewage water, and pond water were inoculated into the biometer flask containing basal-salts medium, ^{14}C-DCPD and nonlabeled DCPD (5 ppm). Part of the DCPD was volatilized and trapped in the KOH solution. $^{14}CO_2$ was found

in the KOH solution of these flasks after CO_2 was precipitated with $BaCl_2$ and retrapped in alkaline solution. The $^{14}CO_2$ evolved during 4 weeks of incubation was about 2 to 3% of the added ^{14}C. More than 90% of the ^{14}C was lost from the media during this period, either through volatilization or through adsorption onto the rubber stopper.

Repeated radiolabeled DCPD experiments in 250-ml or 125-ml modified flasks[3] confirmed that DCPD is biodegraded by microorganisms in North Bog water. However, the process is very slow. The loss of DCPD by volatilization is much faster.

Biodegradation Rate in Water. During the study of DCPD biodegradation, we encountered two main problems. The first, and most important, was the volatility of DCPD. In spite of the relatively high boiling point (166°C) and moderate solubility (> 50 ppm), DCPD was lost very quickly from the aqueous media. Secondly, the DCPD gradually oxidized and produced a yellowish product on the surface when the chemical was stored in the refrigerator or at room temperature. The yellow color also appeared in concentrated DMSO solutions (40 mg/ml) stored at room temperature. Therefore, the reagent DCPD and ^{14}C-DCPD had to be redistilled to increase their purity, and they were stored in the freezer.

Because of the high volatility of DCPD and the very slow DCPD biodegradation, it was difficult to study the rate in a regular, aerated reactor. Measuring the degradation rate by chemical analysis of the disappearance of DCPD was not practical. Only the $^{14}CO_2$-evolution test appeared sensitive enough to detect DCPD degradation. Several attempts have been made to design and conduct a $^{14}CO_2$-evolution rate study, but they were complicated by the need for head space for supplying oxygen to microbes. Furthermore, rubber stoppers in standard reaction flasks were found to adsorb vaporized DCPD and to accelerate its volatilization from the medium.

Finally, a glass reaction vessel was designed (Figure 6). This vessel enabled us to obtain samples of the medium (from below the surface) and from the KOH trap by syringe without venting the system. The liquid basal-salts medium with ^{14}C-DCPD and unlabeled DCPD (5 ppm) was inoculated with acclimated North Bog microorganisms, kept in glass-stoppered flasks, and shaken by hand twice a week. In this experiment, the $^{14}CO_2$ produced in 3 weeks was 1.6% of the ^{14}C added. However, the ^{14}C remaining in the medium contained 68% of the original activity, and the uninoculated control also showed 65% activity remaining after the same period. Presumably, most of the loss was from volatilization during the incubation and sampling--especially during the replacement of the KOH solution, when temporary ventilation is required. The volatilization loss was much larger than the $^{14}CO_2$ evolution.

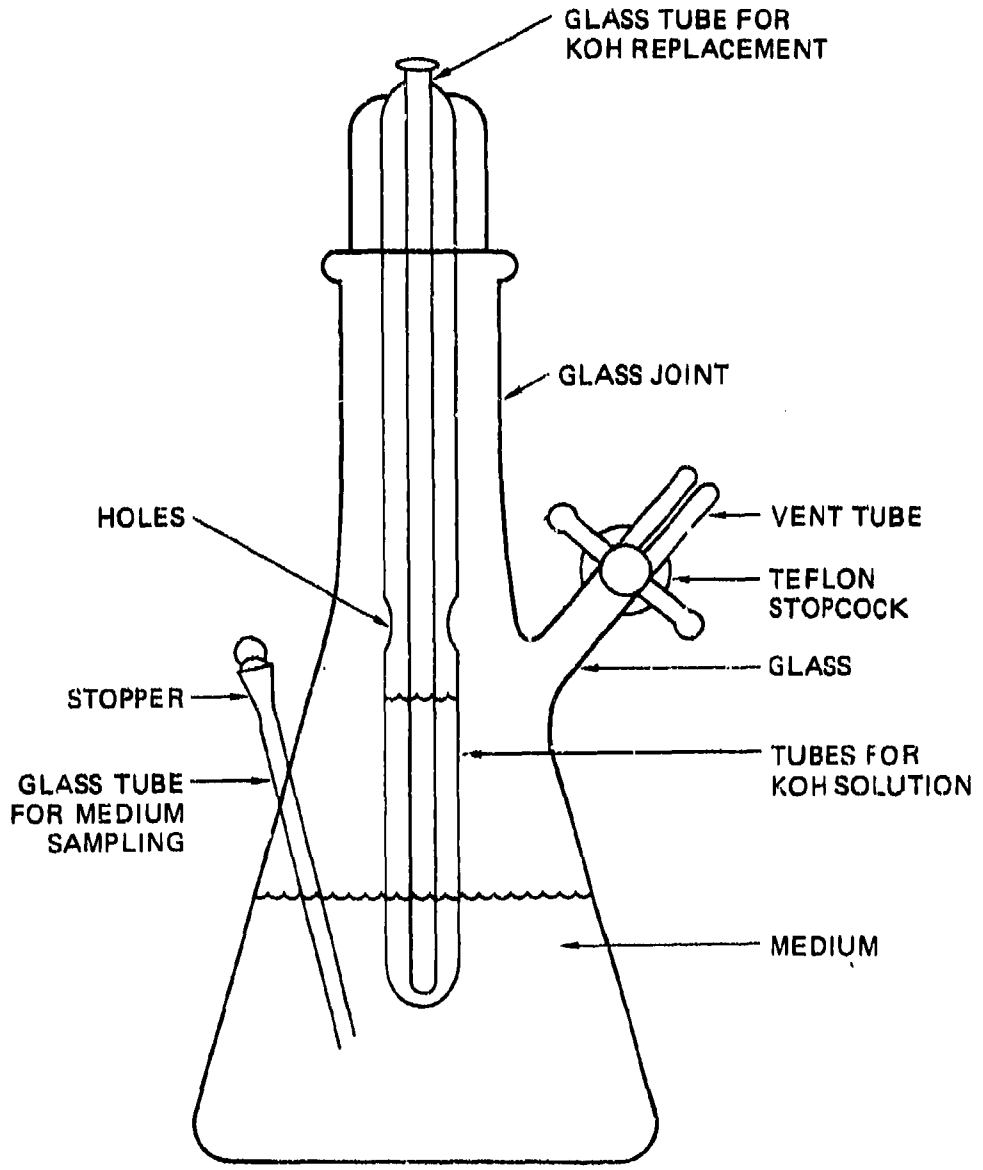


FIGURE 6 APPARATUS TO STUDY BIODEGRADATION OF VOLATILE COMPOUNDS

In another experiment, Trypticase Soy broth (TSB, 500 ppm) was added to basal-salts medium and DCPD. The $^{14}CO_2$ produced was only 0.6% of that added, while a flask without TSB produced 0.5% in 5 weeks. The TSB provided nutrients to increase the microbial cells but did not help the DCPD biodegradation. The dissolved oxygen level in the TSB flask was below 10% of saturation at week 5. High cell concentrations in the flask caused high oxygen consumption and lowered the amount of oxygen available. Lack of oxygen may be a reason why the DCPD biodegradation rate was not increased by TSB addition. The flask without TSB still had more than 80% oxygen. The ^{14}C remaining was 37% in DCPD and 57% in DCPD plus TSB media.

These experiments indicate that without evaporation, $^{14}CO_2$ evolution may take more than 1 year to account for 50% of the added ^{14}C.

To investigate the $^{14}CO_2$ evolution rate with a very high cell population, North Bog microorganisms were grown in TSB medium (6 g/liter) spiked with DCPD, in shaker flasks. The cells at the end of log growth phase were centrifuged, washed, and resuspended in basal-salts medium. The cell count was 4×10^9 cells/ml. Five hundred milliliter of this high-cell-concentration suspension was placed in a 1-liter sealed glass bottle reactor and starved for 3 hours, then ^{14}C-DCPD was added (2.8 µCi, 5-ppm DCPS). The head space was flushed with air for 4 min every hour (200 ml/min), and exhausted gas was bubbled through hexane to remove volatilized DCPD and then bubbled through KOH solution to trap $^{14}CO_2$. No $^{14}CO_2$ was detected during 5 hr of incubation. A control bottle with the same cell suspension showed that the level of dissolved oxygen in the magnetic-bar-stirred bottle was 2 to 8 ppm during the incubation. More than two-thirds of the DCPD was lost during this period, mainly by volatilization, and no metabolites were detected by GC. Because of the volatilization loss and lack of transformation, this method could not be used for a DCPD degradation kinetic study.

Soil Biodegradation. A preliminary test for the ultimate biodegradation of DCPD in soil was initiated with the soil collected in August. To a mixture of soils from Pit #1, Pit #4, and the North Bog, 0.72 µCi of ^{14}C-DCPD was added. The final concentration of DCPD was 0.19 ppm. The weekly KOH trap samples showed that both $^{14}CO_2$ and DCPD were present. The accumulated CO_2 evolution and total ^{14}C trapped during 34 weeks of incubation are shown in Figure 7. The flask with SRI soil also showed that DCPD was degraded. The accumulated $^{14}CO_2$ was 5.4% of the added ^{14}C, and the accmulated total ^{14}C trapped was 9.4%.

Ultimate biodegradation of DCPD in soil was initiated with the RMA soils collected in August (incubated at 25°C) and soil collected during January (incubated at 10°C). Duplicate flasks with 2 levels of DCPD were used for each temperature. To one set of flasks was added 1.4 µCi of ^{14}C-DCPD and DCPD (0.37 ppm) and to another set was added 1.4 µCi of ^{14}C-DCPD and 1.37 ppm of DCPD. To another pair of flasks with higher levels of DCPD, 2 g of 18-week-old preliminary test flask soil was added.

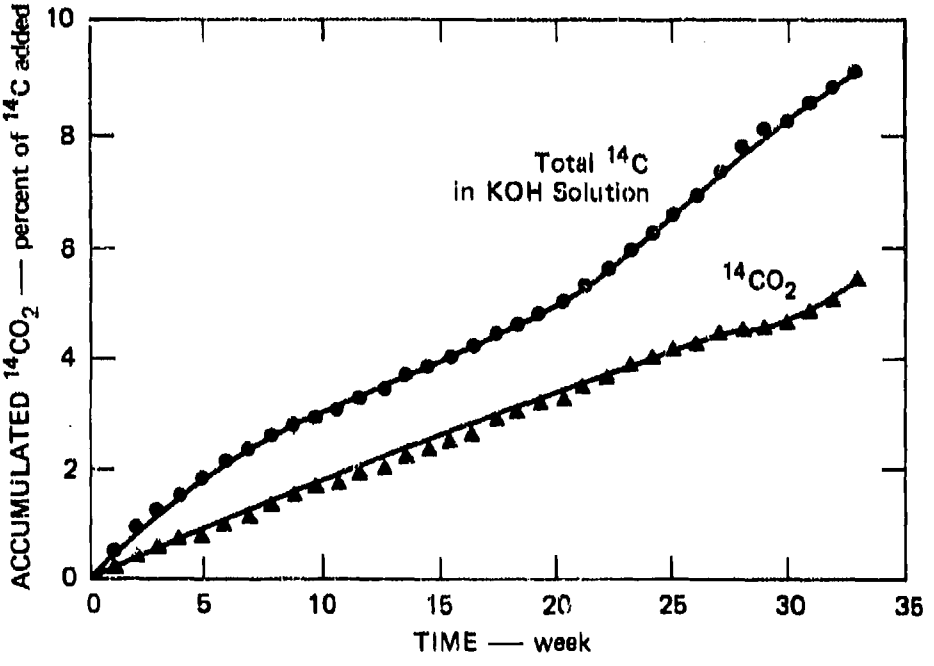


FIGURE 7 ACCUMULATED $^{14}CO_2$ AND TOTAL ^{14}C TRAPPED IN PRELIMINARY TEST FOR DCPD SOIL BIODEGRADATION

The accumulated $^{14}CO_2$ evolved during 17 weeks is shown in Figure 8. The $^{14}CO_2$ evolved from the 2 levels of DCPD is about the same. With the soils inoculated with acclimated soil, $^{14}CO_2$ evolution was higher, producing 4.1% while the flasks without inoculum produced about 1.1% at 25°C during 17 weeks of incubation. The soil incubated at 10°C produced 0.2% $^{14}CO_2$ during this time, about one-fifth the rate of that at 25°C. The accumulated totals of ^{14}C trapped accounted for 1.8% and 0.5% of the added ^{14}C, respectively, at 25° and 10°C. In the flasks with acclimated soil, the total ^{14}C trapped accounted for 7.3%.

3. Volatilization Rate Study

The oxygen reaeration method[15] was used to estimate the rate of DCPD loss from water. First, the rate constants k^{DCPD} and $k_V^{O_2}$ were determined in the laboratory using various stirring rates. They appear in Table 8 and are graphed in Figure 9. The slope of the line, $k^{DCPD}/k_V^{O_2}$, was 0.54. From Equation 2, the rate constant k_V^{DCPD} can be determined for various water bodies, using known oxygen reaeration rate values.[16] For a pond $k_V^{O_2} = 0.008\ hr^{-1}$; for a lake, $k_V^{O_2} = 0.01\ hr^{-1}$.

$$\left[k_V^{DCPD}\right] = \left[k_V^{O_2}\right]_{\text{water body}} \times \left[k_V^{DCPD} \Big/ k_V^{O_2}\right]_{\text{laboratory}} \qquad (2)$$

For the North Bog waters, the estimated rate constant of DCPD is $0.0054\ hr^{-1}$. From this value, the half-life can be estimated as follows:

$$t_{1/2} = \frac{0.693}{k_V^{DCPD}} = \frac{0.693}{0.0054} = 128.3\ hr = 5.3\ days. \qquad (3)$$

4. Analytical Chemistry

The analysis of waters from RMA showed 2-ppb DCPD in the North Bog surface and deep waters collected in August. The January sampling showed 100 ppb. The higher concentration of DCPD in the winter may be the result of the inhibition of volatilization by the ice layer that covers the North Bog. The well water at Pit #4 obtained during the January sampling contained 11.2 ppb DCPD.

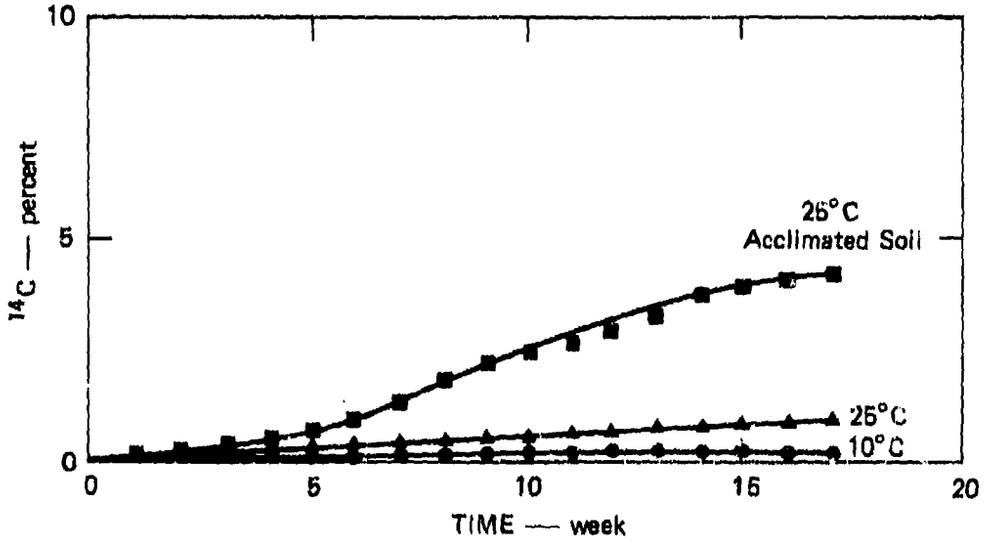


FIGURE 8 ACCUMULATED $^{14}CO_2$ EVOLUTION IN SOIL IN DCPD BIODEGRADATION AT 25°C AND 10°C

Table 8

RATE CONSTANTS FOR DCPD AND O_2
AT VARIOUS STIRRING RATES

Stirring Rate (RPM)	k_v^{DCPD} (hr^{-1})	95% Confidence Limits	r^2	k_v^{O} (hr^{-1})	95% Confidence Limits	r^2
208	0.807	± 0.040	0.990	1.56	± 0.03	0.9999
241	1.29	± 0.08	0.988	1.97	± 0.04	0.9999
308	2.21	± 0.08	0.996	4.40	± 0.23	0.9987
346	2.55	± 0.21	0.980	5.37	± 0.12	0.9996
373	6.05	± 0.27	0.995	10.44	± 0.14	0.9999

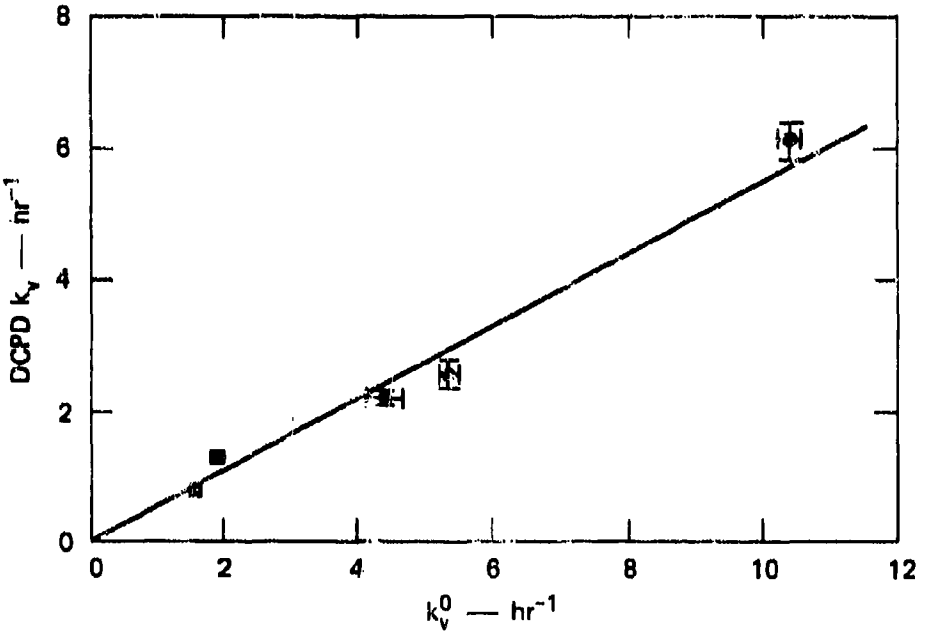


FIGURE 9 RATE OF DCPD LOSS FROM WATER AS A FUNCTION OF REAERATION RATE

D. Conclusions

DCPD will undergo photolysis reactions only in the presence of natural water sensitizers. This indirect method of photolysis, however, occurs very slowly, and a half-life of more than 76 days is estimated.

Biotransformation also occurs very slowly in water and in soil. Because of DCPD's volatility, its half-life is difficult to predict. Any biotransformation in water should be slower than indirect photolysis. This transformation is also very slow in soil. Biodegradation in both soil and water appears to slow with decreasing temperature, and only trace amounts of degradation occur at 10°C. Therefore, any contribution of biotransformation to the total environmental fate of DCPD would occur to the greatest extent in the summer. Rough estimates of biodegradation (to CO_2) half-life would be 4 to 7 years in soil and 1 to 2 years in water at 25°C.

Volatilization appears to be a primary fate of DCPD; the estimated volatilization half-life of 5 days far surpasses those of photolysis and biodegradation. This estimate is based on experiments performed at 25°C, and variations in rate are expected if this half-life is applied to the North Bog at RMA. For instance, in the winter, the North Bog is covered with a layer of ice, which inhibits volatilization. North Bog water had 100 ppb DCPD at this time, while only 2 ppb DCPD was found in August, which probably reflects the effect of volatilization.

E. Recommendations

For further studies on the environmental fate of DCPD, we recommend that the sorption partition coefficient be determined in RMA soils. The leaching of DCPD from soil to water bodies such as the North Bog is probably the rate-controlling process. Lysimeter studies should also be performed to determine the movement in soil. This information, plus the characteristics of the groundwater flow to open water bodies should help define the half-life of DCPD resulting from environmental transport (soil to water to air).

Chemical oxidation might also be considered as an environmental transformation process for DCPD. Distilled samples of DCPD turned yellow on the surface even when refrigerated, in months. GC/MS evaluation of products showed the incorporation of oxygen in the DCPD molecule, indicating that the DCPD had been oxidized. DCPD has allylic hydrogens that might be reactive to free-radical oxidation. Although no evidence of oxidation was observed in the biological or photochemical studies,

the concentrations of the products may have been too low to have been observed. In any event, oxidation of the neat DCPD liquid and dilute aqueous solutions of DCPD are not comparable, since free-radical chains reacting in the neat liquid may have longer chain lengths than those in the aqueous solutions in which free-radical chain-termination reactions may be dominant. We believe, however, that this process cannot compete with volatilization in the removal of DCPD from fresh water.

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